PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/53, 21/45, 21/51 — A1

(11) International Publication Number: WO 91/04489

(43) International Publication Date: 4 April 1991 (04.04.91)

(21) International Application Number: PCT/CA90/00309

(22) International Filing Date: 20 September 1990 (20.09.90)

(30) Priority data:
409,788 20 September 1989 (20.09.89) US

(71) Applicant: THE ROYAL INSTITUTION FOR THE ADVANCEMENT OF LEARNING (McGILL UNIVERSITY) [CA/CA]; 3550 University Street, Montreal, Quebec H3A 2A7 (CA).

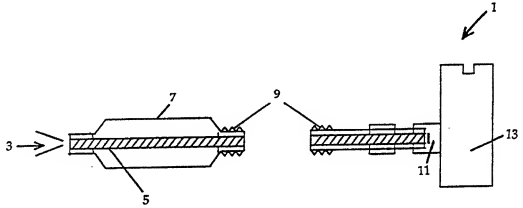
(72) Inventor: ISMAIL, Ashraf, A. ; 4160 Dorchester West, Apt. A01, Westmount, Quebec H3Z 1V1 (CA).

(74) Agent: GOUDREAU GAGE DUBUC & MARTINEAU WALKER; 3400 The Stock Exchange Tower, P.O. Box 242, Victoria Square, Montreal, Quebec H4Z 1E9 (CA).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: A HOMOGENEOUS INTERFEROMETRIC IMMUNOASSAY SYSTEM



(57) Abstract

The present invention relates to a homogeneous immunoassay system for the determination of an antibody or an antigen in a sample which consists of an interferometric signal from an optical source, a waveguide coated with an antibody or an antigen and having at least one region immersed in a solution containing a sample, whereby the corresponding antigen or antibody can be complexed on the waveguide, a detector adapted to measure the interferometric signal after its propagation through the waveguide, and a measuring device to take the Fourier transform of the interferometric signal for determining the degree of attenuation of the interferometric signal at a wavelength corresponding to an absorption characteristic of the antigen-antibody complex or of a label incorporated into the antigen-antibody complex, whereby determining the amount of antigen or antibody in the sample.

* See back of page

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MC	Monaco
AU	Australia	FI	Finland	MG	Madagascar
BB	Barbados	FR	France	ML	Mali
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GR	Greece	NL	Netherlands
BJ	Benin	HU	Hungary	NO	Norway
BR	Brazil	IT	Italy	PL	Poland
CA	Canada	JP	Japan	RO	Romania
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
DE	Germany	LK	Sri Lanka	TD	Chad
DK	Denmark	LU	Luxembourg	TG	Togo
				US	United States of America

TITLE OF THE INVENTION

An homogeneous interferometric immunoassay system.

BACKGROUND OF THE INVENTION

Conventional optical spectrometers are dispersive instruments, employing a prism or a grating to separate the radiation emitted by the source into its component wavelengths, with each resulting spectral element detected individually. As a result, these spectrometers are inefficient in their use of the energy available from the source since, at any given time, only a small fraction of the energy reaches the detector. This inefficiency is particularly disadvantageous in applications which involve low energy throughput, such as attenuated total reflectance (ATR) experiments. Conventional spectrophotometers utilize filters to block all wavelengths except the wavelength band of choice and, accordingly, also utilize only a fraction of the source energy.

A Fourier transform (FT) spectrometer operates on the totally different optical principle of interferometry. An interferometrically coded signal contains information for a range of wavelengths; computing the Fourier transform of the signal yields a spectrum. The first interferometer was built by Michelson in 1891, and his design is incorporated in most commercial FT spectrometers currently in use. A corner cube interferometer is also utilized in some commercial FT spectrometers.

The most important factor, in practice, contributing to the sensitivity of FT optical spectrometry (FTOS) is Fellgett's, or the multiplex, advantage, which gives rise to improvements in signal-to-

noise ratio (S/N) and reduction in measurement time for FT spectral acquisition, compared to the time required to obtain spectra with a grating instrument. Fellgett's advantage originates in the fact that information for all wavelengths emitted from the source reaches the detector at the same time in FTOS measurements, whereas in a grating instrument each spectral element (υ cm^{-1}, corresponding to the resolution) is detected separately. This results in a dramatic decrease in measurement time if information from a large spectral region is required. This is an important consideration if multiple components absorbing in different regions of the spectrum are to be detected.

Another advantage associated with FT spectrometers is Jacquinot's advantage. The magnitude of Jacquinot's advantage is assessed by comparison of the maximum optical throughput permissible before loss of resolution is incurred for a grating spectrometer and an interferometer. In a grating instrument the throughput is significantly limited by the size of the slits, resulting in low throughput. Since there are no slits in an interferometer, more energy impinges on the detector.

The use of a laser in an interferometer to trigger digitization of the signal gives rise to a third advantage, known as Connes' advantage. This refers to the precision (0.003-0.006 cm^{-1}) with which frequencies can be determined in FTOS work, because the laser serves as an internal wavelength calibration standard. Since the digitized spectra are stored as a series of data points corresponding to fractions of laser wavelength, which does not vary with time, spectra recorded at widely separated times can be compared with precision. This is a particularly important advantage for such data handling

techniques as spectral subtraction and coaddition of scans, which can be subject to difficulties when performed on a computerized grating instrument due to drifts in frequency with time.

FTOS spectrometers have been found to be very useful in attenuated total reflectance (ATR) experiments. In an ATR experiment, light from the source is transmitted down a suitable waveguide (the internal reflection element, or IRE), such that it is totally reflected at the IRE-sample interface, giving rise to an evanescent wave which penetrates into the sample. As a result of the attenuation of the evanescent wave by the sample, the light exiting the IRE and striking the detector is attenuated at wavelengths corresponding to absorptions of the sample, thus yielding the spectrum of the sample. Due to the shallow depth of penetration of the evanescent wave into the sample, a short effective pathlength is obtained. However, the use of ATR techniques with conventional spectrometers is limited by the poor signal-to-noise ratio of the spectra obtained. As a result of the advantages of FT spectrometers, as outlined above, the coupling of ATR techniques with FTOS has made possible the acquisition of high-quality spectra. Several manufacturers have designed small ATR cells offering automatic sampling and self-cleaning capabilities, facilitating the routine quantitative analysis of samples.

It would be highly desirable if these techniques could be adapted to an immunoassay system. That is, an interferometrically coded signal from an optical source would be propagated down an optical waveguide, and the resulting attenuation of the evanescent wave would provide a measure of the amounts of an antigen-antibody

complex bound on the outer surface of the waveguide. Such a system would provide a homogeneous immunoassay method that would offer several advantages with respect to existing homogeneous immunoassay methods. The use of a label to mark the antigen-antibody complex would not be a prerequisite in a homogeneous interferometric immunoassay, although the use of such a label would facilitate data interpretation, whereas most existing methods require the labeling of the antigen-antibody complex with an enzyme or a fluorescent tag, the use of each of these labels having its own inherent limitations. Furthermore, such a system would be unaffected by background fluorescence or phosphorescence, unlike existing homogeneous immunoassay systems that employ fluorescent tags.

SUMMARY OF THE INVENTION

Surprisingly and in accordance with the present invention, there is provided an homogeneous immunoassay system for the determination of the presence of an antibody or an antigen in a sample which comprises of:

- an interferometric signal from an optical source;

- a waveguide coated with an antibody or an antigen and having at least one region immersed in a solution containing a sample, whereby the corresponding antigen or antibody can be complexed on said waveguide;

- a detector adapted to measure the interferometric signal after its propagation through the waveguide; and

- measuring device to take the Fourier transform of the interferometric signal for determining the degree of attenuation of the interferometric signal at a wavelenght corresponding to an absorption characteristic of the antigen-antibody complex or of a label incorporated into the antigen-antibody complex, whereby the amount of antigen or antibody in the sample is determined.

Such an homogeneous immunoassay system can be utilized for the determination of any antigen or antibody. The use of a label is not a prerequisite in the general case.

Such an homogeneous immunoassay system can be utilized for the simultaneous determination of multiple analytes in a single homogeneous immunoassay test.

Although the present invention has been described in the foregoing description by way of preferrred embodiments thereof, it should be pointed out that it can be modified at will, within the nature of the present invention.

IN THE DRAWINGS

Figure 1 shows an interferometrically coded signal;

Figure 2 shows a schematic representation of the homogeneous immunoassay system made in accordance with the present invention;

Figure 3 shows a schematic representation of binding of antigen (ag) in solution to their specific antibodies (ab) immobilized on the surface of a waveguide;

Figure 4 shows a homogeneous immunoassay system for detecting antigen (ag) according to the present invention in which antibodies are bound onto the waveguide, and a known amount of labeled antigen (ag*) in the surrounding solution competes for the antibody binding sites with the unlabeled antigen (ag);

Figure 5 shows a homogeneous immunoassay system according to the present invention for the detection of an antibody (ab) in which antigens are bound onto the waveguide, and a known amount of labeled antibodies (ab*) competes for the antigen with the unlabeled antibody;

Figure 6 shows a curve representing the formation of BSA-anti-BSA complex at the surface of the waveguide;

Figure 7 shows 4 curves representing the variation in the extent of complex formation between BSA immobilized on a waveguide and anti-BSA-$X(CO)_3$ in solution surrounding the waveguide: (1) 0.05 mg/ml anti-BSA-$X(CO)_3$; (2) 0.1 mg/ml anti-BSA-$X(CO)_3$; (3) 0.15 mg/ml anti-BSA-$X(CO)_3$; (4) 0.19 mg/ml anti-BSA-$X(CO)_3$ and 0.25 g/ml free BSA.;

Figure 8 shows 2 curves representing intensities of the $\upsilon(CO)$ absorptions of the $X(CO)_3$ label in the ATR spectrum measured from the surface of a waveguide immersed in a solution containing anti-BSA-$X(CO)_3$ (0.3 mg/ml), (a) with a bare waveguide and (b) with a BSA/CML coated waveguide; and

Figure 9 shows superposition of ATR spectra recorded from the surface of a waveguide coated with (a) BSA-$Z(CO)_6$ and (b) BSA-$X(CO)_3$.

DETAILED DESCRIPTION OF THE INVENTION

An embodiment of the homogeneous immunoassay system according to the present invention is shown in Figure 2 and is generally denoted 1. It mainly consists in coupling of an optical fiber waveguide-based measurement cell to an optoelectronic detector assembly in which there are: a lens or beam condenser 3, an optical fiber waveguide 5, an aluminum sample boat 7, a stainless steel optical fiber coupler 9, a detector element 11, and a detector housing 13.

The use of a beam condenser 3 to focus the interferometric signal onto the waveguide 5 aids in increasing the throughput of the signal reaching the detector 11. A variety of beam condensers are commercially available, and simple lens systems can be assembled. Also a detector design based on optical fiber technology has been implemented to enhance signal detection from optical fiber waveguides.

The homogeneous immunoassay system of the present invention is an immunoassay where no separation step is required. In this assay, an optical waveguide coated with an antigen (or antibody) is immersed in a solution containing the corresponding antibody (or antigen). Introduction of the interferometrically coded signal (Figure 1) to the waveguide at such an angle that it is internally reflected through the waveguide establishes an evanescent wave at the waveguide-solution interface. The evanescent wave a the waveguide-solution interface has a depth of penetration proportional to the wavelength of the radiation propagating through the waveguide:

$$d_p = \lambda / \{2\pi [\sin 2\theta - (n_{21})^2]\} 0.5$$

where d_p is the depth of penetration, θ is the angle of incidence, λ is the wavelength of light propagating down the waveguide, and $n_{21} = n_2/n_1$, where n_1 is the refractive index of the waveguide and n_2 is the refractive index of the medium in contact with the waveguide. The evanescent wave is attenuated by absorption of energy by species present within the volume swept out by the depth of penetration.

In order to bind the antigen or antibody on the surface of the waveguide, the waveguide is coated with a cladding on which the antigen or antibody can be adsorbed or covalently bound. The thickness of the cladding on the waveguide can be adjusted so that the depth of penetration is sufficient to sample only the antibody-antigen complex (referred to as the "bound" fraction) on the surface of the cladding with very minimal attenuation of the evanescent wave by the uncomplexed antigen or antibody present in solution (referred to as the "free" fraction).

Coating the waveguide with a cladding, with a thickness less than the depth of penetration, composed of a solid support onto which an antigen or antibody can be adsorbed or covalently linked can be accomplished by a variety of methods. In this invention, the coating of a polymer onto the waveguide to serve as the cladding is carried out by an immersion method, the thickness of the film being controlled by the concentration of the polymer in the solution in which the waveguide is immersed. The polymer employed is a carboxy-modified latex polymer. The thickness of the cladding is estimated from the intensities of the infrared peaks due to absorptions of the polymer film. The antigen or antibody can be adsorbed on the polymer film or it can be covalently linked to the polymer through the carboxyl function.

Other methods of thin film deposition can also be utilized to coat the waveguide with an antigen or antibody.

The underlying principle of immunoassay is that the concentration of the antigen-antibody complex is proportional to the concentration of free antigen and free antibody present in the assay medium. Thus, a calibration curve for the determination of an antigen or antibody can be constructed by measuring the amount of antigen-antibody complex formed upon addition of varying and known amounts of antigen or antibody to a solution containing a fixed and known amount of antibody or antigen. In terms of the present invention, the antigen-antibody complex is formed at the surface of the waveguide (Figure 3), and thus the amount formed can be determined by measuring the attenuation of the evanescent wave, which can be monitored as a function of time or determined at an end point. The attenuation of the evanescent wave is measured at a wavelength corresponding to a characteristic absorption of the antibody-antigen complex. The absorption may be inherent to the antigen-antibody complex or it can arise from a specifically introduced label having a characteristic absorption that can be readily detected. The use of such a label is not a prerequisite to the development of a homogeneous interferometric immunoassay but simply facilitates the data acquisition. Organometallic molecules having characteristic absorptions in the mid-infrared frequency range have been employed thus far as labels in the demonstration of the general viability of a homogeneous interferometric immunoassay. Several different types of homogeneous interferometric immunoassay protocols are possible using the homogeneous immunoassay system of the present invention.

An homogeneous interferometric immunoassay system for the detection of an antigen (ag) has the antibodies bound onto the waveguide, and a known amount of labeled antigen (ag*) in the surrounding solution competes for the antibody binding sites with the unlabeled antigen (Figure 4).

An homogeneous interferometric immunoassay system for the detection of an antibody (ab) has the antigen molecules bound onto the waveguide and a known amount of labeled antibody (ab*) in the surrounding solution competes for the antigen with the unlabeleld antibody (Figure 5).

An homogeneous interferometric immunoassay system for the detection of an antibody (ab) has antibodies of the same type as the antibodies to be assayed bound onto the waveguide and competing with the antibodies in the surrounding solution for labeled antigen molecules in the solution.

A sandwich-type homogeneous interferometric immunoassay for total antibody content is also possible using the system of the present invention. Antigens are coated onto the surface of the waveguide, and antibodies specific to these antigens are introduced in the solution surrounding the waveguide. Following an incubation period, the solution is replaced with a solution containing labeled protein A molecules. Protein A is a substance that binds to the Fc fragment of antibodies. Accordingly, the amount of labeled protein A bound at the surface of the waveguide provides a measure of the total antibody content of the initial solution.

This invention also deals with the first application of a waveguide transmitting radiation in the mid-infrared region of the electromagnetic spectrum for the development of a homogeneous "mid-infrared" immunoassay.

The homogeneous immunoassay system of the present invention can also use the multiplexing advantage of the interferometrically coded signal to detect multiple antigens in a single homogeneous test. Because the interferometrically coded signal covers a wide spectral region, multiple antibodies or antigens having distinct characteristic absorptions can be detected simultaneously. These multiple antibodies or antigens can be coated on the waveguide by several methods. The coating of individual sides of a square waveguide is accomplished by reaction of each side with only one type of antibody or antigen. In this manner, different immunoassays can be performed on the same waveguide. Alternate methods of introduction of multiple antibodies or antigens include serial coating of the antibodies or antigens or the use of optical fiber bundles, with each optical fiber coated with a dfferent antibody or antigen of interest.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

Example I

Homogeneous interferometric immunoassay without the use of a label

The waveguide utilized was a ZnSe rod (8 X 0.5 X 0.5 cm) transmitting radiation in the frequency range between 5000 and 650 cm^{-1}. The waveguide was coated with a thin layer (50-3000 nm) of a

carboxy-modified latex film, either through immersion of the waveguide in a 5-30% (w/v) carboxy-modified latex (CML) paint (from the Seradyn Corporation) or through the evaporation of an aliquot (50-200 ul) of the CML paint applied to the waveguide. After deposition of the CML layer, the coated waveguide was washed 3 times with distilled water and then immersed in 4 ml of a phosphate buffer (0.01 M, pH 7.4) solution containing bovine serum albumin (BSA) (1 mg/ml) and water-soluble carbodiimide [WSC; 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] (1 mg/ml). The waveguide was then washed again 3 times with distilled water and 3 times with buffer. An ATR spectrum was recorded from the surface of the waveguide. The presence of the amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) bands of BSA in the spectrum confirms that BSA is bound to the CML coating on the waveguide.

A solution of anti-BSA (2 mg/ml) in phosphate buffer (0.01 M, pH 7.4) was prepared. Upon immersion of the BSA/CML-coated waveguide in this solution, the binding of anti-BSA to BSA at the surface of the waveguide was monitored by the growth of the amide II band of anti-BSA (1560 cm^{-1}) in the ATR spectrum. A plot of the intensity of the amide II band of anti-BSA versus time exhibited a positive slope up to t = 25 min and then reached a plateau (Figure 6). This behavior is indicative of binding of anti-BSA to BSA until equilibrium is reached and demonstrates that antibody-antigen complex formation at the surface of the waveguide can be monitored by this method, thus establishing its viability as the basis for an immunoassay.

Example II

Homogeneous interferometric immunoassay with the use of a labeled antigen or labeled antibody

Synthesis of Anti-BSA-X(CO)$_3$

Anti-BSA (10 mg) was stirred for 16 h with $HOOCC_6H_5Cr(CO)_3$ (100 mg) in 20 ml of phosphate buffer in the presence of WSC (100 mg) at 4°C. The solution was dialyzed (molecular weight cutoff, 20,000) twice against phosphate buffer and once against water, and the dialysate was evaporated under reduced pressure to yield a yellow solid, anti-BSA-[Lys-ε-$NHCOC_6H_5Cr(CO)_3$]$^{n'}$ henceforth denoted as anti-BSA-X(CO)$_3$. IR (in KBr): ν(CO) 1975, 1910 cm^{-1}.

Synthesis of protein A-X(CO)$_3$

Protein A-[Lys-ε-$NHCOC_6H_5Cr(CO)_3$]$^{n'}$ henceforth denoted as Protein A-X(CO)$_3$, was synthesized by the above procedure. IR (in KBr): ν(CO) 1960, 1900 cm^{-1}.

Synthesis of BSA-X(CO)$_3$

BSA-[Lys-ε-$NHCOC_6H_5Cr(CO)_3$]$^{n'}$ henceforth denoted as BSA-X(CO)$_3$, was synthesized by the above procedure. IR (in KBr): ν(CO) 1969, 1900 cm^{-1}.

Synthesis of BSA-Z(CO)$_6$

To 10 ml of an aqueous solution of BSA (10 mg/ml), $HOOCCCHCO_2(CO)_6$ (100 mg) and WSC (100 mg) were added. The reaction mixture was stirred for 16 h at 4°C and was then dialyzed

against distilled water twice. The dialysate was evaporated under reduced pressure at room temperature to yield a pale reddish-brown solid, BSA-[Lys-ε-NHCOCCCHCO$_2$(CO)$_6$]$_n$, henceforth denoted as BSA-Z(CO)$_6$. IR (in KBr): υ.(CO) 2096, 2057, 2023 cm^{-1}.

Example III

Monitoring of antigen-antibody complex formation in real time

A waveguide coated with CML to which BSA had been covalently bound was immersed in phosphate buffer (0.01 M, pH 7.4). The ATR spectrum from the surface of the waveguide was recorded as the reference spectrum. The buffer was then decanted, and 4 ml of a buffer solution containing labeled anti-BSA-X(CO)$_3$ (0.05 mg/ml) was introduced. Spectral acquisition was commenced immediately upon addition of the antibody solution. The formation of the BSA-[anti-BSA-X(CO)$_3$] complex with time was monitored by the growth of the peaks in the ATR spectrum due to the ν(CO) absorptions of the X(CO)$_3$ label (Figure 7, curve 1). The solution was decanted, and the waveguide was washed 5 times with 15 mM HC1 solution and 5 times with buffer solution. A new reference spectrum was recorded. The experiment was then repeated two times using solutions containing anti-BSA-X(CO)$_3$ at concentrations of (1) 0.1 mg/ml (Figure 7, curve 2) and (2) 0.15 mg/ml (Figure 7, curve 3). In a separate experiment, BSA (0.25 mg/ml) was added in solution together with anti-BSA-X(CO)$_3$ (0.19 mg/ml) to compete against BSA bound at the surface of the waveguide for the anti-BSA-X(CO)$_3$ binding sites (Figure 7, curve 4). The variation in the intensities measured in the ATR spectrum as a function of the concentration of anti-BSA-X(CO)$_3$ in solution (Figure 7, curves 1-3) demonstrates that the signal recorded from the surface of

the waveguide is proportional to the concentration of the labeled antibody in the solution surrounding the waveguide. Furthermore, the decrease in the measured intensities upon addition of BSA in solution to compete against BSA bound at the surface of the waveguide for the binding sites of anti-BSA-$X(CO)_3$ (Figure 7, curve 4) demonstrates the viability of the described technique as the basis for a homogeneous immunoassay.

Example IV

A control experiment was carried out to evaluate the level of background signal in the ATR spectra, i.e. the contribution to the measured intensities of the absorptions of the $X(CO)_3$ label from anti-BSA-$X(CO)_3$ not bound to the BSA on the surface of the waveguide. The background signal can arise in two ways: (1) penetration of the evanescent wave beyond the bound fraction into the solution surrounding the waveguide, and (2) nonspecific binding of anti-BSA-$X(CO)_3$ on the surface of the waveguide. In this experiment, the bare waveguide was immersed in a phosphate buffer solution containing anti-BSA-$X(CO)_3$ (0.3 mg/ml). The ATR spectrum from the surface of the waveguide was recorded and compared to that obtained under the same conditions with the BSA/CML-coated waveguide (Figure 8). The comparison indicates that the maximum contribution from anti-BSA-$X(CO)_3$ not specifically bound at the waveguide to the measured intensities of the $X(CO)_3$ absorptions in the latter spectrum is of the order of 20%. Thus, the measured intensities stem predominantly from BSA-[anti-BSA-$X(CO)_3$] bound at the waveguide surface.

Example V

In order to demonstrate that multiple antigens can be determined simultaneously in interferometric immunoassay, BSA was complexed to two different labels having distinct absorptions. A phosphate buffer solution containing BSA-$X(CO)_3$ and BSA-$Z(CO)_6$ was deposited onto the surface of an anti-BSA/CML-coated waveguide. In the ATR spectrum recorded from the surface (Figure 9), the absorptions of the $X(CO)_3$ and $Z(CO)_6$ labels can be distinguished and their intensities measured independently. Thus, if these labels are complexed to different antigens (or antibodies), the quantitative determination of the two antigens (or antibodies) can be achieved in a single homogeneous immunoassay.

It should be understood, however, that this detailed descrption, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

I CLAIM:

1. An homogeneous immunoassay system for the determination of an antibody or an antigen in a sample which consists of:

- an interferometric signal from an optical source;

- a waveguide coated with an antibody or an antigen and having at least one region immersed in a solution containing a sample, whereby the corresponding antigen or antibody can be complexed on said waveguide;

- a detector adapted to measure the interferometric signal after its propagation through the waveguide; and

- measuring device to take the Fourier transform of said interferometric signal for determining the degree of attenuation of said interferometric signalat a wavelenght corresponding to an absorption characteristic of the antigen-antibody complex or of a label incorporated into the antigen-antibody complex, whereby determining the amount of antigen or antibody in said sample.

2. The homogeneous immunoassay system according to Claim 1, wherein said waveguide consists of a rod.

3. The homogeneous immunoassay system according to Claim 1, wherein said waveguide consists of an optical fiber.

4. The homogeneous immunoassay system according to Claim 1, wherein said waveguide consists of a slide.

5. The homogeneous immunoassay system according to Claim 1, wherein said interferometric signal is used for the detection of multiple analytes in a single test.

6. The homogeneous immunoassay system according to Claim 5, wherein said multiple analytes are labeled with distinct markers or possessing themselves a vibration for distinguishing them from each other and from any species present in the medium.

7. An homogeneous immunoassay method using the system according to Claim 1, comprising the steps of:

a) coating an antigen or an antibody on the waveguide;

b) introducing the corresponding antibody or antigen to the waveguide; and

c) monitoring the formation of antigen-antibody complex on said waveguide.

8. The homogeneous immunoassay method according to Claim 7, wherein a labelled antibody or antigen is added to step b, whereby facilitating the monitoring of the antigen-antibody complex formation.

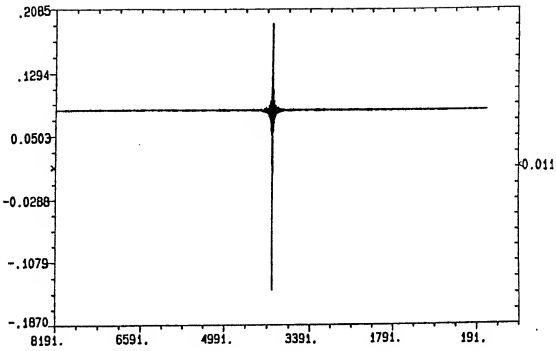

.2085
.1294
0.0503
-0.0288
-.1079
-.1870
8191.
6591.
4991.
3391.
1791.
191.
0.011

Fig. 1

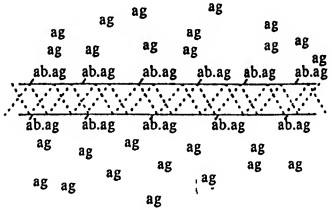

ag
ab.ag

Fig. 3

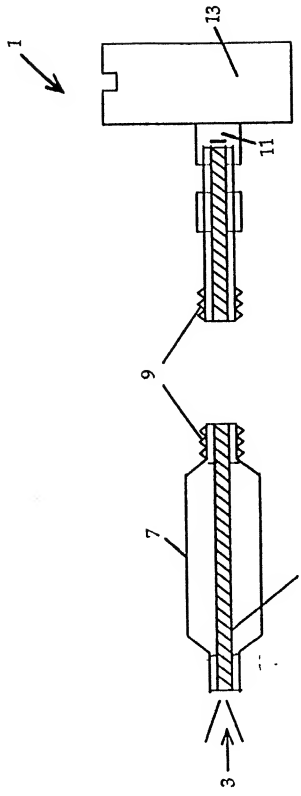


Fig. 2

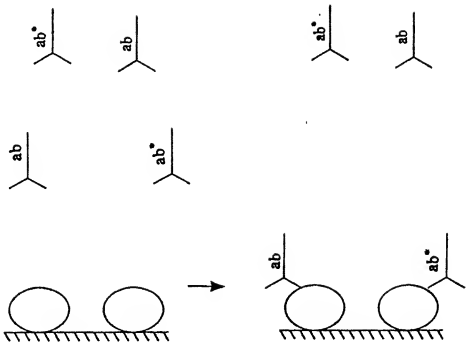


Fig. 5

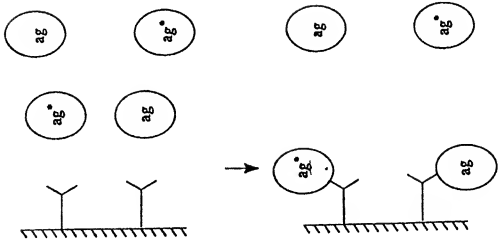


Fig. 4

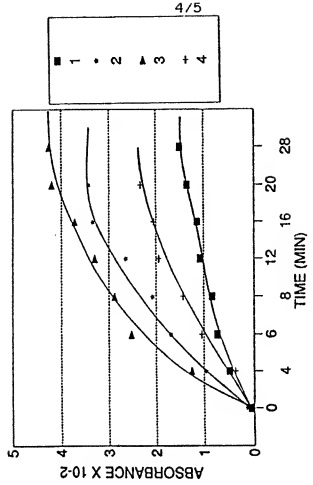

ABSORBANCE X 10-2
0
1
2
3
4
5
0
4
6
8
12
16
20
28
TIME (MIN)
1
2
3
4

Fig. 7

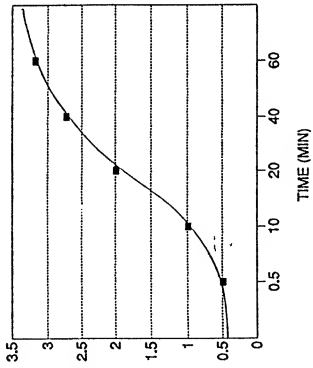

ABSORBANCE X 10-2
0
0.5
1
1.5
2
2.5
3
3.5
0.5
10
20
40
60
TIME (MIN)

Fig. 6

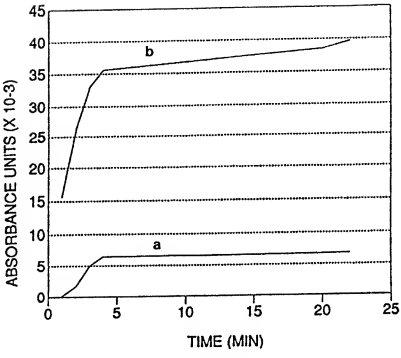

ABSORBANCE UNITS (X 10-3)
TIME (MIN)
a
b
0
5
10
15
20
25
30
35
40
45

Fig. 8

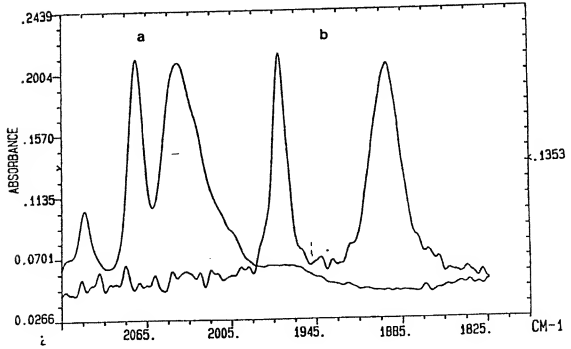

ABSORBANCE
.2439
.2004
.1570
.1135
0.0701
0.0266
.1353
a
b
2065.
2005.
1945.
1885.
1825.
CM-1

Fig. 9